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A Time to Divide: Does the Circadian Clock Control Cell Cycle?

A variety of molecular, genomic and epidemiological evidence has linked cell cycle regulation to circadian rhythms. In a recent issue of *Molecular Cell*, Koeffler and colleagues show that PER1 sensitizes human cancer cells to ionizing radiation-induced apoptosis. In addition, PER1 expression was found to be downregulated in human tumors, suggesting a tumor suppressor role for the PER1 protein. The significance of PERs, clocks, and cell cycle control is discussed.

It is well recognized that organisms sense and respond to daily oscillations in light by altering their physiological processes and activities. This cycling, know as the circadian rhythm, imparts a biological advantage by allowing an organism to keep in step with its changing environment. At the cellular level, these cycles correspond to diurnal fluctuations in the availability of not only nutrients and oxygen, but also to cell stressors and damaging agents. Thus it seems logical that cell division would be linked to circadian rhythms. In support of this idea, transcriptome analysis has revealed that many important cell cycle genes are regulated in a circadian fashion. Such genes include *MYC* (G_0/G_1 transition), *cyclin-D1* (G_1/S transition), and *WEE1* (G_2/M transition) (Fu et al., 2002; Matsuo et al., 2003). These genes function at important regulatory points of the cell cycle, suggesting that the circadian oscillator may control various points of cell division. If this idea is true, then it could provide insight into human proliferative diseases such as cancer.

In addition to the molecular and genomic support for the idea of circadian regulation of the cell cycle, there is epidemiological evidence for a relationship between disruption of circadian regulation and cancer. In this regard, an increased risk for breast cancer has been reported among female night shift workers (Hansen, 2001). Similarly, patients with colorectal cancers who exhibited normal daily rhythms had longer survival compared to those with disrupted circadian rhythms (Mormont et al., 2000). In addition, studies investigating the timing and efficacy of cancer treatments ("chronotherapy") indicate that the status of the circadian oscillator can influence cellular responses to genotoxic stress induced during chemotherapy (Gorbacheva et al., 2005). This influence is thought to have a metabolic component, but may also involve a sensitivity of cancer cells at particular times of the circadian day. Taken in sum, there are indications from both in vitro and in vivo studies that support the connection between circadian rhythms and cancer sensitivity.

Circadian oscillations in gene expression are reliant upon a feedback loop of transcriptional activation and repression. Central to this loop is the CLOCK/BMAL1 (MOP3) heterodimer that acts in the positive arm of the circadian cycle by transactivating clock-controlled genes (CCGs) such as the *Period* (*PER1-3*) and the *Cryptochrome* (*CRY1-2*) genes. The products of the *PER* and *CRY* genes play a role in the negative arm of the circadian cycle to inhibit CLOCK/BMAL1 activity. The PER components aid in nuclear localization of the PER/CRY complex, while the CRY components appear to function mainly as transcriptional repressors. Additional controls over this feedback loop include the CCG product, casein kinase 1ε, which is involved in regulating the rates of PER and CRY degradation through posttranslational modification. The cyclical nature of *BMAL1* expression in part drives the circadian oscillation and may be positively regulated by other CCGs, *RORA*, and negatively regulated by *Rev-erb-*α. This second interlocking loop is thought to maintain the precise and robust nature of rhythms in mammals.

In a recent issue of *Molecular Cell*, Koeffler and colleagues (Gery et al., 2006) explored the relationship between *PER1* expression and response of human cancer cells to ionizing radiation (IR). They found that overexpression of *PER1* sensitized cancer cells to IR-induced apoptosis and that this phenotype was associated with altered expression of key cell cycle regulators, including *MYC* and *p21*. The *MYC* locus is a CCG whose expression is suppressed by the CLOCK/BMAL1 complex. The finding that IR treatment induced *PER1* expression and nuclear localization suggested a mechanistic link between circadian cycling and cell growth control. Since PER proteins play a role in inhibiting BMAL1 activity, it was proposed that high levels of PER1 could result in decreased activity of BMAL1, leading to derepression of *MYC* and induction of apoptosis. In addition to its influence over IR-induced apoptosis, *PER1* was also found to be involved in pathways that respond to DNA damage, including the ATM kinase and downstream effector, *CHK2*. These factors are activated by double-strand breaks and are involved in initiating DNA repair and cell cycle arrest or apoptosis. PER1 was found to physically interact with and activate components of the ATM pathway, suggesting that it also has a role in the DNA damage response pathway that may be independent of its role in clock function.

Perhaps the most intriguing aspect of the work by Koeffler and colleagues is the relationship between *PER1* expression and cancer cell physiology. In one avenue of experimentation, *PER1* overexpression in cancer cells lead to reduced colony formation and clonogenic expansion. This observation was used as evidence to support the idea that *PER1* suppresses the growth and transforming potential of cancer cells and might act as a tumor suppressor. In a second avenue of experimentation, *PER1* expression was examined in human tumors and it was found to be significantly downregulated in lung and breast tumor tissue compared to matched normal tissue. Taken together, these results suggest that *PER1* is a potential tumor suppressor gene and its downregulation could play a role in tumorigenesis.

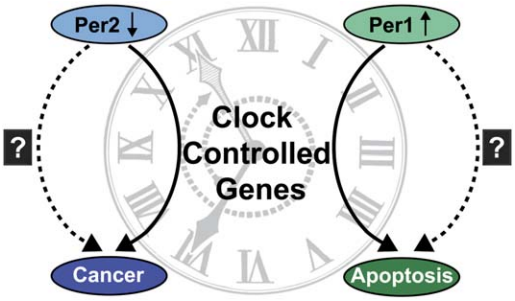

Figure 1. Clocks, Clock-Controlled Genes, and Cell Cycle Decisions

Altered expression of *Period* genes (Per2 deficiency or Per1 overexpression) appears to mediate cell fate decisions (cancer, apoptosis) through regulation of the expression of various clock-controlled genes involved in cell cycle regulation and DNA damage control. Whether the *Period* genes, known core circadian clock components, act through disruption of the circadian clock or through a clock-independent mechanism (question marks) is still unclear.

The data regarding *PER1* and tumorigenesis are interesting especially in light of the earlier observation that *mPer2* null mice are unusually cancer prone, developing spontaneous salivary gland hyperplasia and teratomas much sooner than their wild-type counterparts (Fu et al., 2002). In wild-type mice, clock genes are upregulated and *Myc* expression is repressed following γ-radiation. These responses fail in the *mPer2* null mice, leading to derepression of *Myc* and uncontrolled cell growth and tumor formation. The combined findings of *PER1* overexpression inducing apoptosis and *mPer2* deficiency resulting in uncontrolled cell growth are intriguing and suggest a tumor suppressor role for the *PER* genes and support the idea that disruption of a circadian factor may increase risk for carcinogenesis (Figure 1).

Although the results of the *PER* experiments can be interpreted in light of circadian biology, they are not definitive proofs of such a link. In this regard, recent data in the literature demonstrated that mice with a complete disruption of circadian rhythms do not display an increased sensitivity to cancer. Using the $Cry1^{-/-}Cry2^{-/-}$ murine model, cell cycle and DNA damage check-points, as well as predisposition to spontaneous and IR-induced cancers, were found to be no different than in wild-type mice (Gauger and Sancar, 2005). These results have lead Sancar and colleagues to support the view that disruption of circadian rhythm, in itself, is not sufficient to sensitize cells to cancerous transformation. Rather, it may be that individual clock components, such as the PERs, play independent roles in other biological processes, such as the cell cycle. That is, PER may act outside the framework of the circadian oscillator by mechanisms that are yet to be determined.

The link between PER function and cell cycle control and tumorigenesis is an intriguing one. Perhaps one of the most important questions going forward is whether it is circadian biology that is directly linked to cell division and DNA damage control or whether it is individual components of the clock that take on unique roles in these biological processes. Arguing for a direct link between circadian clocks and cell cycle is the *PER1* and *Per2* evidence cited above, as well as the observation that $Cry1^{-/-}Cry2^{-/-}$ mice display reduced rates of mitosis and lagging liver regeneration after partial hepatectomy (Matsuo et al., 2003). Arguing against the importance of a link between circadian rhythms and cell division is the unaltered sensitivity of $Cry1^{-/-}$ $Cry2^{-/-}$ null mice to spontaneous and IR-induced cancer, as well as the observation that most circadian mutants, including *Bmal1(Mop3)* and *Clock* mutants, as well as *Per1/Per2* and *Cry1/Cry2* double mutants, display apparently normal embryogenesis. Hopefully, experimental evidence in the years to come will reveal how clocks themselves, or the individual clock components, are causally linked to proliferative diseases such as cancer. A better understanding of the function of clock proteins in the biology of cell division and cancer could be an important first step in the development of new strategies to prevent and treat human malignancy.

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